

## BIOCHEMICAL COMPOSITION OF SOYBEAN SEEDS

### [*GLYCINE MAX* (L) MERRILL] AS AFFECTED BY SULPHUR NUTRITION

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#### ABSTRACT

*The present investigation was carried out to evaluate the effect of different levels and sources of sulphur on physiological parameters and biochemical composition of soybean seeds. The experiment was laid in Randomized Block Design with three replications in two cropping seasons, 2011 and 2012. There were seven treatments, control, gypsum and single super phosphate (SSP) at three levels 10, 20 and 30 kg S ha<sup>-1</sup>, respectively. Sulphur fertilization increased dry matter, 1000 seed weight and seed yield of soybean in both the years of study. Maximum seed yield was observed with gypsum at 30 kg S ha<sup>-1</sup> in 2012. Protein quality parameters viz. total soluble proteins, free amino acids and sulphur containing amino acids (cysteine and methionine) were significantly increased, whereas trypsin inhibitor activity decreased with different sulphur treatments. Glycinin, a sulphur rich sub-fraction of soybean seed storage proteins, maximally increased with gypsum at 10 kg S ha<sup>-1</sup>. Electrophoretic analysis followed by densitometry of 2-propanol soluble proteins revealed decrease in relative percentage of bands corresponding to protease inhibitors such as Kunitz inhibitor and Bowman Birk type inhibitor. The reduction in N:S ratio with sulphur application indicated more sulphur uptake and increased nitrogen utilization efficiency in protein synthesis. The results suggest that sulphur nutrition as gypsum or SSP resulted in improvement of seed quality parameters in soybean.*

**KEYWORDS:** Soybean, Protein Quality, Sulphur Containing Amino Acids, Gypsum, Seed Storage Proteins

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#### INTRODUCTION

Soybean is being cultivated in India as an important source of high quality protein and oil (Hosmath *et al*, 2014) and consistently provide a cost competitive source of protein for the animal industry and human consumption. The major storage proteins in soybean are globulins consisting of two sub-fractions glycinin and  $\beta$ -conglycinin accounting for 60-70% of total storage proteins. In addition, there are other known bioactive proteins, including the Kunitz trypsin, Bowman-Birk and related protease inhibitors,  $\beta$ -amylases, lipooxygenases, urease and seed lectins which account for about 2-5% of the total seed protein in soybeans (Liener, 1994).

Glycinins are rich in sulphur containing amino acids but the overall sulphur amino acid content of these proteins is still not sufficient to meet the nutritional demands of humans. Plant sulphate assimilation and sulphur amino acid (cysteine and methionine) synthesis are of nutritional importance for animals that lack this capability (Tabe and Higgins, 1998; Hoefgen *et al*, 2001). Cysteine is the first committed molecule formed in plant metabolism containing both sulphur and nitrogen (Wirtz *et al*, 2004). Cysteine serves as a precursor for synthesis of chemically active biological compounds including glutathione, biotin, CoA and enzymes involved in redox reactions (Leustek *et al*, 2000). Synthesis of cysteine is a culmination of carbon, nitrogen and sulphur metabolism

and regulation of network among these pathways determine the nutritional quality of soybean seeds (Hawkesford and de Kok, 2006). Sulphur deficiency has been reported to influence composition of seeds thus impacting nutritional quality (Ahmed *et al*, 2005). Sulphur is an essential element and plays a pivotal role in various plant growth and development processes and is the constituent of sulphur containing amino acids, methionine (21% S) and cysteine (27% S) and other metabolites such as glutathione (Devi *et al*, 2012). Sulphur may enhance seed formation and protein quality and its structure stabilization by forming disulphide bridges (Hell, 1997). The seeds of soybean are the most vital and crucial input for crop production and improvement of quality parameters in mature seeds is highly imperative. Balanced fertilizer practice is required to mitigate the impact of sulphur deficiency and to increase the sulphur use efficiency. Keeping this in view, investigation on 'effect of sulphur application on soybean' was conducted at Punjab Agricultural University, Ludhiana, India.

## MATERIALS AND METHODS

Soybean var. SL 525 was grown in experimental area of Plant Breeding and Genetics, PAU, Ludhiana to study the effect of sulphur fertilization on biochemical composition of seeds. The experiment was laid in randomized block design with three replications in two cropping seasons 2011 and 2012 with seven treatments i.e. control, gypsum and single super phosphate (SSP) each at three different levels 10, 20 and 30 kg S ha<sup>-1</sup> respectively. Each block was divided into seven unit plots for seven treatments. Thus, the total number of plots under the experimental design were 21. The field and the plots were of sizes 21.7 m × 17.4 m and 5 m × 2.7 m respectively. Each plot comprised of 6 rows which were 45 cm apart. The spacing between the blocks was 1.2 m. The soil of each plot was uniformly fertilized with urea as a nitrogen source and rock phosphate as phosphorus source. In calculating the amount of phosphorus, its content in SSP was reduced from the rock phosphate. Fertilizers were applied at the time of final land preparation as basal dose.

Seeds were harvested at maturity and subjected to analysis after achieving constant dry mass. Physiological parameters viz. dry matter, 1000 seed weight and grain yield were recorded. Biochemical composition viz. total soluble proteins (Lowry *et al*, 1951), total free amino acids (Lee and Takahashi, 1966) and glutathione (Habig *et al*, 1974) was estimated. Sulphur containing amino acids (cysteine and methionine) were extracted by hydrolysis of seed powder with 0.2 M Tris HCl (pH 7.2) containing 0.002 M EDTA and 0.5% 2-mercaptoethanol and 25 mg of enzyme papain at 50°C for 4 h. Cysteine and methionine were estimated by the method of Gaitonde (1967) and Horn *et al* (1946) respectively. Free sugars were extracted with 80% and 70% alcohol on boiling water bath and contents of total soluble sugars (Dubois *et al*, 1956) and sucrose (Roe *et al*, 1934) were estimated. Total nitrogen (McKenzie and Wallace, 1954) and total sulphur content (Chesnin and Yien, 1950) were determined by the standard procedures already reported in literature. Trypsin inhibitor activity was determined by the method of Kakede *et al* (1974). 2-propanol soluble proteins were extracted with 60% 2-propanol, centrifuged and supernatant was mixed with three volumes of acetone and incubated overnight at -20°C. Precipitated proteins were re-suspended in SDS sample buffer (Krishnan, 2005) and electrophoretically separated following the method of Laemmli (1971) on 12.5% running gel (w/v) at 25 mA for 3 h. The protein bands were visualized with Coomassie Brilliant Blue G-250 and assessed for their molecular weight by running standard marker proteins (from Genei, India; MW 18-97 KDa). Quantitative assessment of relative content was made by computer assisted densitometry using Quantity-1 software (Biorad) and protein was reported as relative amounts per gel. Globulins were fractionated into glycinin (11S) and β-conglycinin (7S) by repeated isoelectric precipitation at pH 6.4 (glycinin) and 4.6 (β-conglycinin) (Basha and Beevers, 1975). Protein fractions were recovered by centrifugation, lyophilized and their

contents were determined by the method of Lowry *et al* (1951). Data was analysed using Analysis of Variance and critical difference (CD) was evaluated among different treatments.

## RESULTS AND DISCUSSIONS

Dry matter content of seeds indicated higher dry matter content with gypsum @ 20 kg S ha<sup>-1</sup> and 30 kg S ha<sup>-1</sup> in year 2011 and 2012 respectively as compared to control as well as lower doses of gypsum/SSP. Thousand seed weight increased significant in year 2012 with highest 1000 seed weight with SSP @ 20 kg S ha<sup>-1</sup> in both the cropping seasons. Sulphur applied in the form of gypsum at different dose rates significantly increased seed yield as compared to control in 2011, but values were statistically similar among different doses of gypsum whereas in 2012, gypsum and SSP significantly increased grain yield to maximum with 30 kg S ha<sup>-1</sup> as compared to control (Table 1).

**Table 1: Effect of Sulphur Application on Physiological Parameters of Soybean.**

Treatments		Dry Matter (g/100g)		1000 Seed Weight (g)		Grain Yield (kg ha <sup>-1</sup> )	
		2011	2012	2011	2012	2011	2012
Control		96.03±0.45	93.69±0.13	93.23±2.28	99.66±0.51	1962	2500
Gypsum	10	96.16±0.25	93.85±0.16	93.53±4.14	107.87±4.82	2204	2513
	20	97.96±0.15	94.43±1.30	98.13±3.86	105.25±3.56	2284	2527
	30	96.80±0.98	95.91±2.28	94.50±0.78	108.49±2.87	2231	3138
SSP	10	95.86±0.58	94.64±1.08	98.70±7.66	109.39±3.39	1935	2611
	20	96.20±1.00	94.68±1.06	99.13±5.87	109.99±2.37	2069	2680
	30	96.86±0.45	94.83±1.00	96.50±2.29	105.36±2.21	2123	3097
CD (P<0.05)		1.20	1.29	NS	2.38	213	309

Data is represented as mean±S.D. of three replications

Sulphur applied in the form of gypsum at different dose rates significantly increased seed yield as compared to control in 2011, but values were statistically similar among different doses of gypsum whereas in 2012, gypsum and SSP significantly increased grain yield to maximum with 30 kg S ha<sup>-1</sup> as compared to control. Increase in these yield attributes (dry matter content, seed yield and 1000 seed weight), indicated positive effect of sulphur nutrition on vegetative growth. In earlier studies by Mohanti *et al*, 2004, application of sulphur at 30 kg S ha<sup>-1</sup> produced the highest seed yield in soybean.

## BIOCHEMICAL PARAMETERS

Total soluble proteins increased significantly with application of different levels and sources of sulphur in case of gypsum as compared to control in both seasons (Table 2). Maximum protein content was observed with SSP as sulphur source at 20 kg S ha<sup>-1</sup>. The percent increase in total soluble protein content varied in the range of 3.09 to 5.45% in 2011 and 2.03 to 6.58% in 2012 with different treatments of sulphur supplementation. Gypsum at 30 kg S ha<sup>-1</sup> and SSP at 20 and 30 kg S ha<sup>-1</sup> significantly increased free amino acids in soybean seeds in year 2011. Application of different doses of sulphur fertilizers resulted in significant variations in total free amino acid content, as compared to control in year 2012 with maximum amino acid content with both the fertilizers at 30 kg S ha<sup>-1</sup>.

Cysteine and methionine content increased significantly with gypsum and SSP with increasing doses of fertilization. The increase in protein content due to sulphur application was mainly because of synthesis of sulphur containing amino acids. Ghalotra *et al* (2007) observed that gypsum at 40 kg S ha<sup>-1</sup> was most effective in increasing seed sulphur containing amino acids in *Cicer arietinum*. Similar observations were made in *Vigna radiata* by Kumar *et al* (2013). The thiol of cysteine and glutathione is involved in redox cycle by two thiol↔disulphide conversions and this

change is versatile for redox control and mitigation against oxidative stress in pulses (Leustek and Saito 1999). Sulphur supply significantly increased glutathione content in mature seeds (Table 2).

**Table 2: Effect of Sulphur Application on Protein Quality Parameters in Soybean Seeds.**

Treatments		Total Soluble Proteins (Mg/Seed)		Free Amino Acids (Mg/Seed)		Cysteine (µg/Seed)		Methionine (Mg/Seed)		Glutathione (Mg/G)	
		2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Control		18.20±0.69	26.68±1.24	0.14±0.01	0.23±0.02	52.0±5.29	144±6.0	0.27±0.01	0.26±0.01	0.54±0.03	0.84±0.01
Gypsum	10	19.36±0.69	31.43±0.67	0.15±0.01	0.28±0.01	75.0±3.60	164±4.0	0.28±0.02	0.32±0.01	0.72±0.09	0.91±0.03
	20	20.62±1.76	31.20±1.30	0.15±0.02	0.32±0.01	80.0±4.35	208±8.0	0.30±0.03	0.34±0.01	0.90±0.09	0.90±0.03
	30	20.56±0.59	33.74±0.67	0.18±0.02	0.35±0.01	88.34±2.08	236±6.0	0.38±0.02	0.43±0.02	0.92±0.09	0.82±0.02
SSP	10	20.0±0.93	31.67±1.70	0.15±0.01	0.31±0.01	81.34±4.72	184±9.0	0.34±0.02	0.35±0.01	0.86±0.12	0.80±0.02
	20	22.02±1.08	35.42±2.04	0.17±0.01	0.34±0.01	82.67±5.13	197±6.5	0.39±0.03	0.39±0.01	0.79±0.14	0.92±0.02
	30	22.0±1.92	35.10±1.64	0.21±0.01	0.35±0.01	91.67±3.05	198±2.0	0.41±0.04	0.42±0.01	0.81±0.13	0.90±0.02
CD (P<0.05)		2.03	2.70	0.02	0.02	7.90	11.80	0.05	0.03	0.14	0.04

Data is represented as mean±S.D. of three replications

Sulphur fertilization did not show significant variations in oil content in both cropping seasons except that gypsum at 10 and 20 kg S ha<sup>-1</sup> significantly increased in year 2011. Maximum percent increase in total oil content was 2.33 and 0.88% in year 2011 and 2012, respectively, with gypsum at 20 kg S ha<sup>-1</sup>. Insignificant effect of different doses of sulphur on oil content in soybean seeds has been reported by Farhad *et al* (2010) whereas Gokhale *et al* (2005) reported that application of 30 kg S ha<sup>-1</sup> increased oil content in soybean seed over control. Soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with seed production.

**Table 3: Effect of Sulphur Application on Total Soluble Sugars, Sucrose, Total Oil Content, TIA and 2-Propanol Soluble Proteins in Soybean Seeds**

Treatments		Total Soluble Sugars (mg/seed)		Sucrose (mg/Seed)		Total Oil (%)		Trypsin Inhibitor Activity (mg/g)		2-Propanol Soluble Proteins (Relative %)
		2011	2012	2011	2012	2011	2012	2011	2012	2012
Control		4.24±0.34	3.87±0.28	3.19±0.35	3.06±0.06	18.35±0.57	19.88±0.53	122.5±5.84	134.5±5.5	11.76
Gypsum	10	4.76±0.36	5.30±0.31	3.19±0.32	3.37±0.10	19.67±0.33	19.04±1.25	106.0±5.29	120.0±5.0	9.95
	20	5.15±0.63	4.28±0.23	4.08±0.16	3.42±0.07	20.68±0.53	20.76±0.03	97.16±4.31	116.0±3.0	9.71
	30	4.97±0.21	4.48±0.25	3.96±0.36	3.86±0.02	17.63±0.30	19.32±0.94	94.33±6.50	91.5±4.5	10.64
SSP	10	4.73±0.29	5.01±0.42	3.85±0.41	4.36±0.10	17.75±0.43	20.60±0.76	116.5±4.76	107.5±2.5	10.69
	20	5.10±0.25	5.11±0.26	4.00±0.09	4.47±0.11	18.94±0.50	19.58±0.46	103.0±5.89	106.5±3.5	8.37
	30	4.99±0.28	4.91±0.09	3.93±0.40	4.72±0.08	18.25±0.56	19.09±0.36	98.66±2.36	94.5±2.5	8.52
CD(P<0.05)		NS	0.50	0.55	0.13	0.81	NS	8.52	6.77	

Data is represented as mean±S.D. of three replications

Significant increase in total soluble sugars with sulphur fertilization was observed during second cropping season and maximum increase in soluble sugars was with gypsum 10 kg S ha<sup>-1</sup>, as compared to control. Sucrose content also increased significantly with both fertilizers as compared to control, although insignificant variability was observed among treatments. Increased sugar content due to sulphur fertilization might have provided enough carbon source and energy for protein and oil synthesis. The level of soluble sugar content can be a sign of supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha *et al* 2001).

Nitrogen content did not vary significantly among various treatments whereas sulphur content increased significantly in soybean seeds with maximum content observed in treatment with SSP at 20 kg S ha<sup>-1</sup> among all the treatments. This resulted in decreased N:S ratio in seeds treated with various doses of gypsum and SSP.

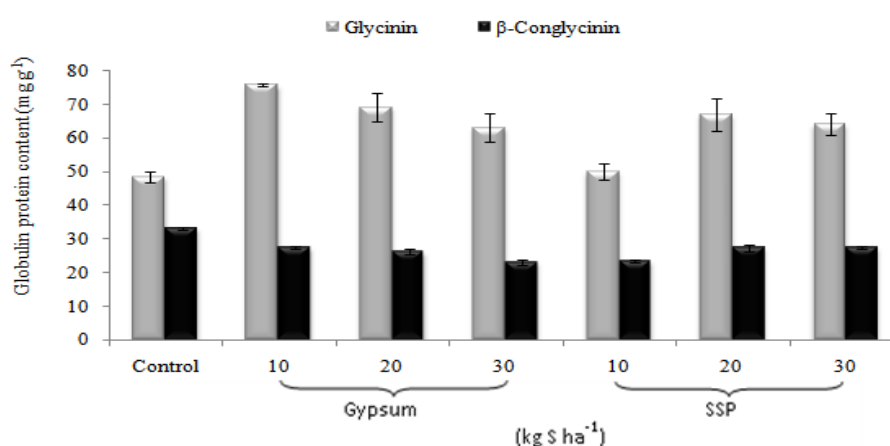
**Table 4: Effect of Sulphur Application on Nitrogen and Sulphur Content in Soybean Seeds**

Treatments		Nitrogen Content (mg/seed)	Sulphur Content ( $\mu\text{g}/\text{seed}$ )	N:S
Control		4.51 $\pm$ 0.13	86.47 $\pm$ 8.27	49.56
Gypsum	10	3.61 $\pm$ 0.62	101.55 $\pm$ 3.5	30.0
	20	3.25 $\pm$ 0.90	118.9 $\pm$ 7.51	24.22
	30	3.27 $\pm$ 0.84	95.1 $\pm$ 8.70	26.81
SSP	10	3.82 $\pm$ 0.75	113.6 $\pm$ 7.80	31.90
	20	4.35 $\pm$ 0.90	133.6 $\pm$ 6.75	28.38
	30	4.20 $\pm$ 0.44	103.68 $\pm$ 5.86	34.86
CD (P<0.05)		NS	13.46	

Data is represented as mean  $\pm$  S.D. of three replications

Since N:S ratio is an indicator of legume quality (Eppendorfer 1971), decrease in N:S ratio in present study suggested increased sulphur uptake and increased nitrogen utilization in protein accumulation. Decreased N:S ratio with sulphur and nitrogen treatments in *Vigna radiata* seeds (Kumar *et al* 2013) and *Cicer arietinum* (Ghalotra *et al* 2007) was also reported earlier.

Both gypsum and SSP significantly increased glycinin content in mature seeds with maximal increase in treatment with gypsum at 10 kg S ha<sup>-1</sup>, in comparison to control seeds as well as other treatments. The increase in glycinin content showed significant decreasing trend with increasing dose rate of gypsum, but values were still higher as compared to control. Sulphur supplementation either in the form of gypsum or SSP significantly decreased  $\beta$ -conglycinin content in seeds.  $\beta$ -conglycinin protein decreased in dose dependent manner with increasing dose of gypsum whereas among various doses of SSP maximum decrease in  $\beta$ -conglycinin was observed with SSP at 10 kg S ha<sup>-1</sup>. Sulphur nutrition influenced expression of sulphur rich relative to sulphur poor seed storage protein genes of legumes (Chandler *et al* 1983, Grabau *et al* 1986). In present study, glycinin content increased whereas  $\beta$ -conglycinins decreased with given sulphur treatments. Gayler and Sykes (1985) reported 40% decrease in the level of glycinins and a contrasting elevation in the level of  $\beta$ -conglycinins with sulphur deficiency in soybean.

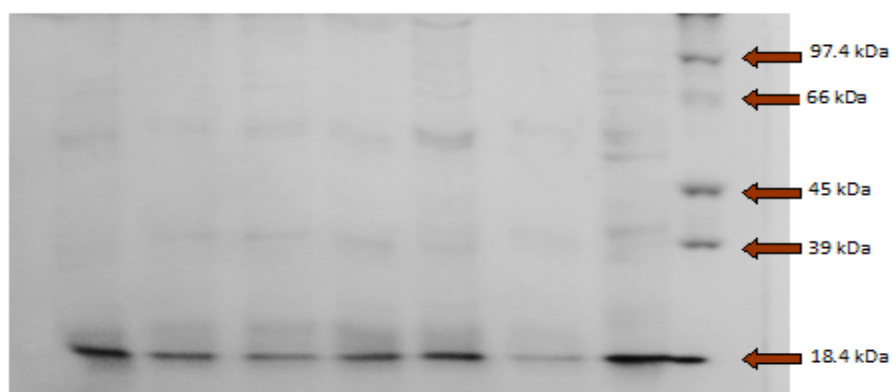


**Figure 1: Effect of Sulphur Application on Globulin Protein Fractions (Glycinin and B-Conglycinin) in Soybean Seeds**

Sulphur deficiency also depressed the synthesis of particular sulphur rich proteins in both *Lupinus albus* (Gillespie *et al* 1978) and *Pisum sativum* (Randall *et al* 1979) that was due to specific repression of the levels of legumin mRNA. Increase in dose rate of sulphur in the form of either gypsum or SSP significantly reduced trypsin inhibitor activity (TIA) in both the years. Minimum TIA was observed in seeds treated with gypsum at 30 kg S ha<sup>-1</sup>. Sulphur fertilizers has

increased the protein content by enhancing protein fractions other than protease inhibitors. Vollmann *et al* (2003) also reported 15% decrease in TIA with the combined application of nitrogen and sulphur or nitrogen alone.

Trypsin inhibitors also play a significant role in determining the protein quality of soybean seeds as these anti-nutritional compounds interfere with human digestion (Guillamon *et al* 2008)). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) pattern of 2-propanol extracted proteins also revealed reduction in the accumulation of these low molecular weight proteins, enriched in Kunitz trypsin and Bowman Birk inhibitor with sulphur fertilization. The maximum relative proportion (11.76 %) of 2-propanol soluble proteins was in control seeds. Both gypsum and SSP at 20 kg S ha<sup>-1</sup> reduced proportion of trypsin inhibitor to 9.71 and 8.37 % respectively.



**Figure 2: SDS-PAGE Profile Of 2-Propanol Soluble Proteins, Resolved On 12.5% Gel. C: Control; 1: Gypsum at 10 Kg S Ha<sup>-1</sup>; 2: Gypsum at 20 Kg S Ha<sup>-1</sup>; 3: Gypsum at 30 Kg S Ha<sup>-1</sup>; 4: SSP at 10 Kg S Ha<sup>-1</sup>; 5: SSP At 20 Kg S Ha<sup>-1</sup>; 6: SSP At 30 Kg S Ha<sup>-1</sup>; M: Protein Marker**

## CONCLUSIONS

Sulphur fertilization improved nutritional composition by enhancing total soluble proteins, sulphur containing amino acids (cysteine and methionine) and sulphur rich glycinin sub-fraction of storage proteins and subsequently lowering the TIA in soybean seeds. Sulphur fertilization improved the assimilation and nitrogen use efficiency as indicated by enhanced protein accumulation rather than soluble nitrogen accumulation in mature seed. Decreased nitrogen to sulphur ratio depicts the improvement of legume quality. Oil biosynthesis has also been affected by sulphur application. Enhanced oil content in mature seeds as compared to control, indicated the involvement of sulphur in formation of sulphhydryl linkages and activation of enzymes responsible for oil biosynthesis. It has been concluded that soybean can efficiently use sulphur and can be used as component of balanced fertilization for nutritional enrichment.

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